

UNIVERSITY OF CALIFORNIA, SAN DIEGO

**Microscopic, Genetic, and Biochemical Characterization of
Non-Flagellar Swimming Motility in Marine Cyanobacteria**

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Marine Biology

by

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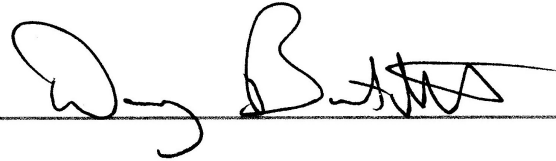
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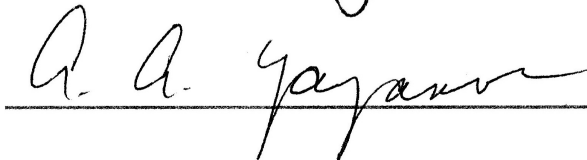
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DEDICATION

To Alex

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ABBREVIATIONS

| | |
|-----------------|---|
| ABC transporter | ATP binding cassette transporter |
| ATP | Adenosine triphosphate |
| BLOTTO | Bovine lacto-transfer technique optimizer |
| CM | Cytoplasmic membrane |
| CMi | Cytoplasmic membrane inner face |
| EDTA | Ethylenediaminetetraacetic acid |
| EL | External layer |
| FITC | Fluorescein isothiocyanate |
| FL | Fibrillar layer |
| HSP | High-speed pellet containing insoluble OM proteins |
| HSS | High-speed supernatant containing soluble OM proteins |
| IgG | Immunoglobulin G |
| MFP | Membrane fusion protein |
| MSCRAMMS | Microbial surface components recognizing adhesive matrix molecules |
| MWCO | Molecular weight cut-off |
| OM | Outer membrane |
| OMP | Outer membrane protein |
| ORF | Open reading frame |
| PAGE | Polyacrylamide gel electrophoresis |
| PAS stain | Periodic acid-Schiff stain |

| | |
|---------------|---|
| PBS | Phosphate buffered saline |
| PCR | Polymerase chain reaction |
| PPIase | Peptidyl-prolyl isomerase |
| Prot1E family | Protein-1 exporter family |
| RSCU | Relative synonymous codon usage |
| RTX | Repeats in toxin |
| S-layer | Surface layer |
| SAPS | Statistical analysis of protein sequences |
| SN | Natural seawater based medium |
| SOW | Synthetic ocean water |
| TOF | Time of flight |
| TEM | Transmission electron microscopy |
| WH8102 | <i>Synechococcus</i> sp. strain WH8102 |

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Publications

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- McCarren, J., J. Heuser, R. Roth, N. Yamada, M. Martone, and B. Brahamsha. 2005. Inactivation of *swmA* results in the loss of an outer cell layer in a swimming *Synechococcus* strain. *J. Bacteriol.* 187: 224-230.
- Palenik, B. B. Brahamsha, F. W. Larimer, M. Land, L. Hauser, P. Chain, J. Lamerdin, W. Regala, E. E. Allen, J. McCarren, I. Paulsen, A. Dufresne, F. Partensky, E. A. Webb, and J. Waterbury. 2003. The genome of a motile marine *Synechococcus*. *Nature* 424:1037-1042.

Conference Participation and Awards

- Wenner-Gren Foundations International Symposium “Marine cyanobacteria: evolution, function and genomes”. Poster titled: Identification and characterization of SwmB, an unusual protein that is required for non-flagellar swimming in marine *Synechococcus* (2005)
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- VIIth Cyanobacterial Workshop. Poster titled: SwmA forms an additional envelope layer in motile marine *Synechococcus* (2001)

ABSTRACT OF THE DISSERTATION

Microscopic, Genetic, and Biochemical Characterization of Non-Flagellar Swimming Motility in Marine Cyanobacteria

by

Jay William McCarren

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2005

Bianca Brahamsha, Chair

The mechanism of motility in marine *Synechococcus*, which swim without any apparent extracellular appendages, remains a mystery 20 years after its discovery. A multifaceted investigation including direct microscopic visualization, genetic analyses, and biochemical approaches was carried out in order to better understand the physiology of this globally important primary producer. Ultrastructural analyses provided a detailed view of the cell envelope layers and aided in the identification of a structure important for motility. Electron microscope tomographic reconstructions

revealed the even distribution of SwmA, a protein required for motility, across the cell surface. Various cryo-fixation techniques were required for the preservation and visualization of a para-crystalline S-layer formed by this protein.

As complete genomic sequence information failed to identify genes involved in motility, a transposon mutagenesis technique was developed to identify components of the motility apparatus. Utilizing this genetic tool, 17 independent transposon insertions that abolish motility were localized to clusters in three separate chromosomal regions. Included within these clusters are several multicomponent transport systems, as well as a number of glycosyltransferases. One cluster is characterized by DNA with an exceptionally low % G+C content relative to the genome average. Additionally, inter-genome comparisons reveal the absence of this stretch of DNA in two non-motile strains of *Synechococcus*, suggesting acquisition of this genetic information by horizontal gene transfer. Contained within this region of low % G+C content is an extremely large gene called *swmB*, which is required for motility in these cells. The sequence of SwmB is highly repetitive, with 4 domains of tandem repeats comprising over 60% of the protein. Analyses confirm that this gene is indeed translated into a megadalton-size protein, which is localized on the cell surface. Cellular localization of the two motility proteins SwmA and SwmB revealed that all motility mutants in culture have a defect in the localization of either SwmA or SwmB and in some instances both of these proteins. Additionally, two outer membrane polypeptides of 70 kDa and 80 kDa are absent in some of these mutants, suggestive of a role in motility.